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Ketone Body Metabolic Enzyme OXCT1 Regulates Prostate Cancer Chemoresistance

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14. ABSTRACT Analysis of needle biopsy samples revealed that OXCT1 was upregulated in a subset of patients and the upregulation was associated with chemotherapy resistance. In vitro analysis showed that OXCT1 was overexpressed in various prostate cancer cell lines compared to benign prostate cells. OXCT1 stable knockdown cell lines in C42B, DU145 and PC3 were established. The optimal docetaxel doses and treatment time were determined. OXCT1 knockdown cells showed lower proliferation and higher sensitivity to docetaxel treatment. Cellular metabolic endpoints such as ATP and ROS were altered by ketone body supplementation. These results confirmed our hypothesis that OXCT1 plays important role prostate cancer chemotherapy sensitivity.					
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1. Introduction

Ketone bodies are important alternative energy source other than glucose and fatty acids. The role of ketone body utilization pathway in regulating prostate cancer resistance to docetaxel-based chemotherapy has never been tested. OXCT1 encodes the rate limiting enzyme converting ketone bodies to acetyl-CoA. The goal of this project is to investigate the role of OXCT1 in prostate cancer chemotherapy sensitivity and the molecular mechanism.

2. Keywords

chemosensitivity, OXCT1, docetaxel, metabolism

3. Overall Project Summary

Current Objectives

To determine the role of OXCT1-mediated ketone body utilization in regulating prostate cancer cell response to docetaxel, cellular metabolism, and redox balance.

Results

Analysis of prostate cancer needle biopsy samples revealed that the OXCT1 gene was overexpressed in nearly 50% of patients (1). Kaplan-Meier survival analysis indicated that higher OXCT1 level was associated with lower relapse free survival rate (Fig. 1). This suggests that OXCT1 overexpression or ketone body utilization pathway may promote prostate cancer cells malignancy and mediate prostate cancer chemotherapy resistance.

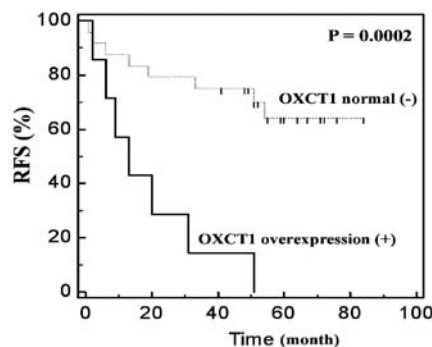


Figure 1. Relapse-free survival (RFS) based on pre-treatment OXCT1 mRNA in LCM-cancer compared to matched benign epithelium. n=31.

To confirm the findings on cellular level, we examined OXCT1 levels in different prostate benign and cancer cell lines. We found that OXCT1 was expressed in several prostate cancer cell lines and that the level of OXCT1 was higher in prostate cancer cell lines compared to benign cells (Fig. 2).

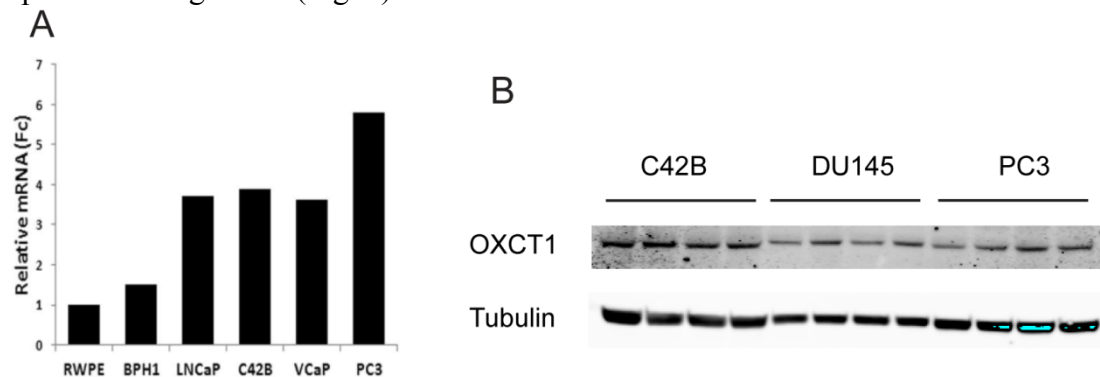


Figure 2. A. OXCT1 mRNA in prostate benign (RWPE and BPH1) and cancer cell lines. B. OXCT1 protein in prostate cancer cell lines.

To investigate the role of OXCT1 in docetaxel chemosensitivity, we established OXCT1 stable knockdown cell lines (Fig. 3A) and measured the proliferation rate in prostate cancer cells. The proliferation rate was lower in OXCT1 knock down cells (shOXCT1) than that in non-target control knockdown cells (shC, Fig. 3B).

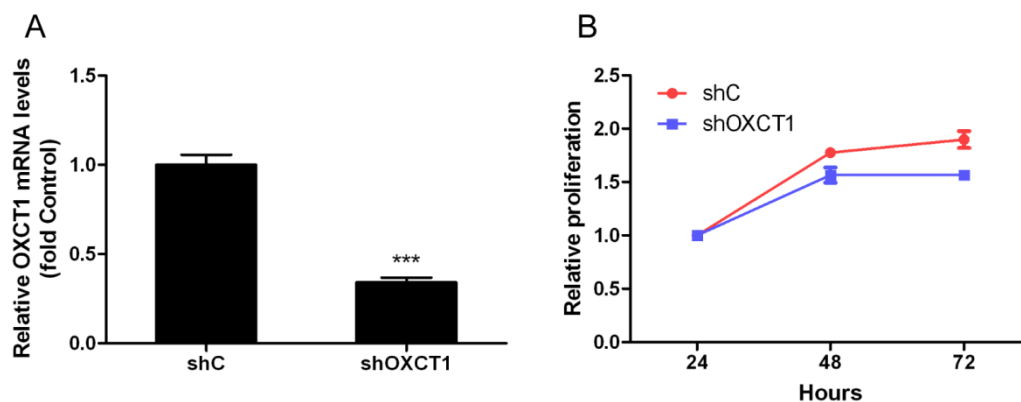


Figure 3. A. mRNA levels of OXCT1 with non-target control (shC) and OXCT1 (shOXCT1) stable knockdown. B. Proliferation of prostate cancer cells. ***p<0.001.

Then to determine the optimal dose of docetaxel, we treated C42B and DU145 cells with 0-40 nM docetaxel. The results showed that in C42B cells with 0-5 nM docetaxel treatment showed significant decrease in cell viability; however, in DU145 cells, which are more resistant, 5-40 nM docetaxel treatment showed significant decrease in cell viability (Fig. 5).

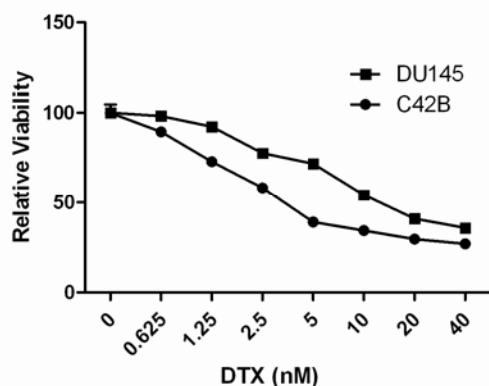


Figure 5. Optimal docetaxel (DTX) doses for cell viability in C42B and DU145 cells.

Then we examined docetaxel sensitivity in prostate cancer cell lines and determined the optimal treatment time of docetaxel. The results showed that OXCT1 knockdown cells had lower cell viability compared to non-target control knockdown cells with docetaxel treatment after 48h and 72h (Fig. 6), indicating that cells with lower OXCT1 levels are more sensitive to docetaxel treatment.

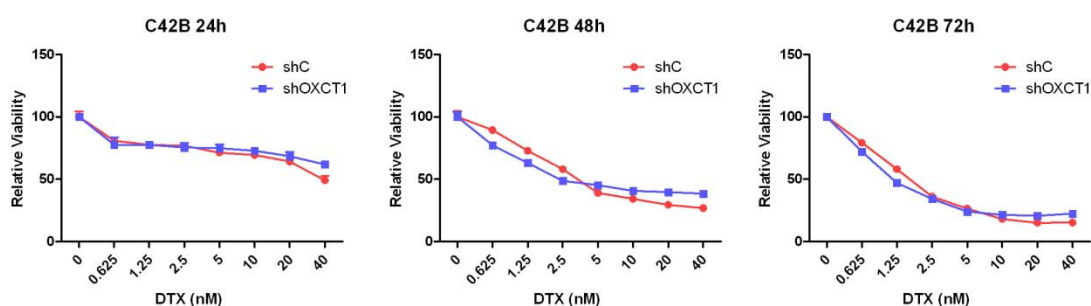


Figure 6. Cell viability was measured in C42B cells after different docetaxel treatment time. shOXCT1 cells are more sensitive to docetaxel treatment.

Using the optimal docetaxel doses and treatment time determined above, we observed similar results in PC3 cells (Fig. 7A), which showed increased docetaxel sensitivity after OXCT1 knockdown. We further analyzed the effect of ketone body on cellular metabolism.

Treatment of PC3 cells with acetoacetate increased cellular ATP levels (Fig. 7B) and decreased reactive oxygen species (ROS) levels (Fig. 7C). These results indicated that ketone bodies/OXCT1 play a role in regulating prostate cancer cell energy homeostasis.

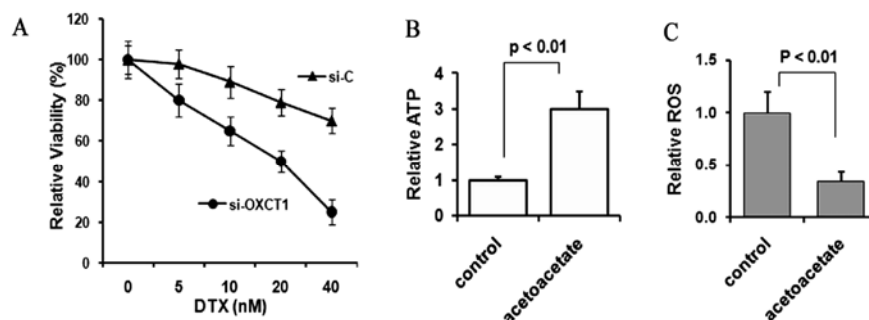


Figure. 7 A. Cell viability in PC3 cells after 48h docetaxel treatment. Cells were treated with 1mM acetoacetate for 24h, then B. ATP and C. ROS levels were measured.

Progress and Accomplishments

We tested OXCT1 levels in different prostate cancer cell lines and established OXCT1 stable knockdown prostate cancer cell lines. The optimal doses of docetaxel and ketone bodies were determined. Cell viability was determined in OXCT1 knockdown cells with docetaxel treatment. The results revealed that decreased OXCT1 levels were associated with higher docetaxel sensitivity. Cell proliferation was measured and lower proliferation was observed in OXCT1 knockdown cells. Cellular Metabolic endpoints ATP and ROS levels were measured under ketone body supplementation conditions.

Discussion

Analysis of our previous data from patient needle biopsy samples indicated that higher OXCT1 levels are associated with docetaxel chemotherapy resistance. In order to investigate the role of OXCT1 in prostate cancer chemotherapy resistance, we used different prostate cancer cell lines with distinct resistance mechanisms. For example, PC3 and DU145 cells without androgen receptor are highly resistant to docetaxel compared to LnCap and C42B cells. The optimal docetaxel dose to measure cell viability was lower for C42B and compared with that for DU145, which was in consistency with their different sensitivity. The optimal docetaxel treatment time was determined to be 48h, when shC and shOXCT1 cells showed obvious different sensitivity. The cells death may be saturated under longer treatment time. OXCT1 is a key enzyme in ketone body metabolism and

cellular metabolism. Knocking down of OXCT1 decreased cell proliferation, while ketone body altered cellular metabolic endpoints such as ATP and ROS. These results suggested that knocking down OXCT1 interfered with ketone body metabolism and altered cellular metabolism as well as cell growth, thus increasing docetaxel sensitivity. However, detailed mechanisms are under further investigation in this project.

4. Key Research Accomplishments

- a. OXCT1 level in prostate cancer cell lines was validated and stable cell lines were established.
- b. The hypothesis that OXCT1 regulates prostate cancer cell chemosensitivity to docetaxel was confirmed in prostate cancer cell lines.
- c. We observed that OXCT1 and ketone body play an important role in cellular metabolism and cell proliferation.

5. Conclusion

The current data confirms the hypothesis that OXCT1 regulates prostate cancer cell chemotherapy resistance, suggesting a new potential therapeutic strategy to improve prostate cancer chemotherapy. Future work will be further characterizing the underlying mechanisms and regulatory networks.

6. Publications, Abstracts, and Presentations

Nothing to report

7. Inventions, Patents and Licenses

Nothing to report

8. Reportable Outcomes

- a. OXCT1 levels are upregulated in a subset of prostate cancer patients and prostate cancer cell lines.

b. OXCT1 upregulation was associated with prostate cancer chemotherapy resistance.

9. Other Achievements

OXCT1 stable cell lines were developed.

10. References

1. Qian DZ, Huang CY, O'Brien CA, Coleman IM, Garzotto M, True LD, Higano CS, Vessella R, Lange PH, Nelson PS, Beer TM. Prostate cancer-associated gene expression alterations determined from needle biopsies. Clin Cancer Res 15 (9): 3135-42, 2009.

11. Appendices

None.

12. Training and Development Opportunities

I have frequent one-on-one work with mentor, which improved my scientific skills including experimental and analytical as well as scientific writing skills. I attended AACR (American Association for Cancer Research) 2014 annual meeting with the support of this grant, which helped to extend my professional networks and research resources. I also participated in various seminars in OHSU. I attended workshops for professional development such as applying for jobs and grant writing offered by OHSU, which greatly helped to develop my career.